THE HISTOLOGY OF THE SKIN LESIONS IN VARICELLA.

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Although varicella is generally regarded as a disease of childhood, the following report deals entirely with the skin lesions in adults. These cases were observed among adult male Filipinos, inmates of Bilibid Prison, at Manila, P.I.

Concerning the prevalence of this disease in the islands I could obtain but scanty information. In some localities it is recognized by the natives as a distinct disease, and is called in the Tagalog " buluton tubig" or waterpox, to distinguish it from " buluton " or smallpox. In one of the provinces a native boy, with face pitted from a former attack of smallpox, was met with who showed the characteristic eruption of varicella.

The occasional occurrence of varicella in adults is generally accepted, although some eminent authorities claim never to have seen a case. Thomas [15, 16] states that he never saw a case in an adult, and that eruptions resembling varicella in adults indicate variola. Von Genser [6] has analyzed 29,250 cases of varicella, and finds that 98.22 per cent occurred before the fifteenth year of life, and 1.78 per cent between the fifteenth and sixty-first year. Race, climate, and confinement in crowded prisons doubtless contribute to make adults more susceptible to this disease. In the cases dealt with in this paper the diagnosis was definitely established by inoculation experiments, and by the histology of the skin lesions as well as by the clinical features of the disease.

It was formerly a matter of controversy whether variola and varicella are etiologically identical, and but different

NOTE. — The work on which this paper is based was carried on in the Government Biological Laboratory at Manila. It was undertaken in connection with an investigation upon smallpox which Dr. W. R. Brinckerhoff and I were carrying on at the time. I am indebted to Dr. Brinckerhoff for many valuable suggestions, and for the interest which he gave to this work. The opportunity for this study of varicella was afforded through the courtesy of Dr. Moulden, physician to Bilibid Prison. Funds were furnished by a grant of two Bullard Fellowships.

manifestations of one and the same infection. Hebra and certain others of the Vienna School have maintained that the two diseases are identical, but most modern authorities are agreed that variola and varicella are two distinct diseases, produced by different infecting agents. The chief data bearing on this question, which the following cases present, are the occurrence of vaccination or smallpox previous to the varicella attack. In a study of thirty-eight cases of varicella, three had the face deeply pitted by a previous attack of smallpox, and of this trio two also bore scars from recent vaccinations. Of the remaining thirty-five cases, thirty-two bore either recent or both old and recent vaccine scars. There is no record of vaccine scars in the other three cases, but it is not improbable that they had been recently vaccinated, for a general vaccination had been accomplished some time before these observations on account of the occurrence of smallpox in the prison. If the two diseases are identical, as asserted by Hebra, it is difficult to explain why the severe form as seen in variola vera, as well as the oft-repeated vaccinations, should not protect against so slight a form as varicella.

Varicella is recognized as an easily communicable disease, yet less is known concerning the mode of infection than is the case with smallpox. While some authorities believe that the disease is communicable from a day or so previous to the eruption up to the time of complete healing of the skin lesions, the presence of the infectious agent in the skin lesions has not been conclusively proved by experimental inoculation.

Inoculation of children with the vesicle contents of varicella has been tried in many instances, but there is great discrepancy in the results obtained. Heim,[7] Vetter,[20, 21] Thomas,[15, 16] Czarkert,[2] Fleischmann,[3, 4] Buchmüller,[1] Smith,[12] and others were unable to produce the disease by inoculation. Fleischmann, from his first series of inoculations, concludes that it is not possible to produce either variola or varicella by the inoculation of varicella lymph. In seven inoculations done at a later date he obtained a general eruption in one case and a local reaction in two. Buchmüller gives a

series of thirty inoculations with varicella. There was some local inflammation in children so inoculated, and in one case a general varicella eruption. He regarded this as being a chance infection rather than the result of the inoculation.

Opposed to these results are those of Hesse,[8] Steiner,[13] d'Heilly,[8] and others. Hesse made inoculations with varicella in one hundred and fifteen cases, which resulted in a local reaction in seventeen, in a general eruption in nine, and was without result in eighty-seven cases. Hesse inoculated with purulent material and dried crusts as well as with clear lymph. Both Vetter and Thomas consider Hesse's results of doubtful value: (1) on account of the possibility that his positive results were due to the use of smallpox lymph instead of varicella lymph; (2) some cases may have been due to spontaneous infection. Vetter asserts that the inoculations should be done with the perfectly clear lymph of typical varicella vesicles, and should not be done during an epidemic but from sporadic cases of varicella. Eimer, who has critically considered Hesse's work, concludes that only one of his cases can be accepted.

Steiner's results are, in the two cases which he gives in detail, beyond question. He inoculated two children, one four years old and previously vaccinated, the other two years and not vaccinated. Neither had had varicella previous to this time. He inoculated the upper arm with perfectly clear lymph from the vesicles of a case which he considered undoubted varicella. Four days after the inoculation there was elevation of temperature which increased, accompanied by some constitutional disturbance, up to the eighth day when a typical varicella eruption appeared. The development of the eruption together with the symptoms he describes in full. The parallelism of the two cases, as regards time of onset and eruption relative to inoculation, makes spontaneous infection seem improbable. He had previously inoculated eight children with varicella with positive results in six, making a total of eight successful inoculations. Of the eight cases five had been previously vaccinated, so that it seems improbable that he dealt with a mild form of variola.

The data furnished by d'Heilly's inoculations are meager. He claims to have produced an eruption in two of the cases inoculated, one after an incubation of three, the other after an incubation of seventeen days.

We see by this review that the attempts to produce varicella by inoculation show positive results in but few cases, and some of these are not beyond question.

The only animal inoculations which I have found recorded are those of Freyer[5] and those of Park.[11] Freyer collected the fluid contents of twenty grayish-yellow, clouded vesicles, and ground it in a mortar after adding a small amount of glycerine. With this mixture he inoculated a calf without result. The calf was inoculated afterward with vaccine lymph and reacted in a typical manner. Park inoculated two monkeys with varicella material with negative results. He points out the diagnostic value of this proceeding, and cites a case in which it was used.

The only account of the histological changes in the skin lesions in varicella is that of Unna.[18, 19] He takes up the changes occurring in the epidermis in the development of the vesicle, and describes two types of cell degeneration which he designates "reticulating colliquation" and "ballooning" degeneration. His description of these may be summarized briefly as follows:

1. Reticulating colliquation affects chiefly the older cells of the epidermis, that is the stratum spinosum and outer layers. The cells increase in size, and in stained sections appear pale. This swelling may be followed either by a partial or total solution of the interior of the cell. The cell membrane, however, persists so that large cellular cavities are formed. If the softening of the interior of the cell is partial a coarse reticulum will be found, but if the softening is more complete the cell will appear as a hollow chamber within which is usually a small amount of granular coagulum lying either against the nucleus or against the cell wall. By the liquefaction of the interiors of cells with the persistence of the cell walls, a reticular appearance is produced. Both nucleus and cell wall finally react to certain stains in a manner similar to fibrin.

2. Ballooning degeneration affects the cells at the base of the vesicle, that is the Malpighian layer. The cells increase in size, lose their dense periphery and protoplasmic bridges. The nuclei become swollen, lose their network, the chromatin collects at the periphery, and direct nuclear division begins. He points out that in this case the stimulation to divide is from within the nucleus, and the centrosome and cytoplasm takes no part. Complete cell division is prevented by the early coagulation of the cytoplasm. Through this type of direct nuclear division, which takes place rapidly, large multinuclear cells are formed. Later in the process, material taking the diffuse stain appears within cells undergoing this form of degeneration, and finally both cytoplasm and nucleus stain diffusely. Thus cells presenting the ballooning as well as those presenting the reticulating types of degeneration finally undergo a "fibrinoid" transformation. In varicella lesions the ballooning degeneration is a more prominent feature than the reticulating colliquation. The formation of the vesicle is accounted for by the penetration of fluid exudate into the epidermis entering into or separating degenerated cells and forming spaces in the tissue which enlarge and tend to coalesce to form a single chamber.

In this paper I report thirty-eight cases of varicella occurring in adult male Filipino prisoners.

There had occurred in the prison about three hundred cases at the time the last observations were made, and new cases were continuing to appear sporadically. Attention was first directed to them for the purpose of establishing a diagnosis. Several cases of smallpox had occurred in the prison previous to the appearance of this disease so that it was at first important to determine whether a mild form of smallpox or a different disease was present. Later on a further study of the disease was made. This consisted of the inoculation of animals with the exudation from the skin lesions, and of a study of the pathology of the skin lesions.

In two cases first seen a few lesions were present scattered over the surface of the body. These consisted of elevated,

flat-topped pustules which possessed a central crust; this in some cases was slightly depressed. They contained a whitish, semi-fluid exudate, evidently pus. There was but little induration at the base of the pustule, and the entire process was quite superficial. The exudate was collected from several of these lesions and used to inoculate one monkey and two rabbits. All inoculations were made immediately after the collection of the exudate.

Monkey No. 1. — The skin of the belly was shaved and several shallow scratches were made and inoculated with the exudate mentioned above. Four days after the inoculation the surface of the skin was elevated and slightly opaque at certain points along the scratches. One of these elevations was excised for histological study. Six days after inoculation there were small crusts situated upon the remaining scratches. These crusts were used to inoculate the corneas of Rabbit No. 4. Nine days after inoculation, three small papules, two millimeters in diameter, were present in the vicinity of the inoculation scratches. One such papule was excised for histological study.

About a week subsequent to the last observation the monkey was inoculated with lymph from a case of variola in man and typical variola lesions developed.

Stained paraffin sections of the skin excised four days after inoculation show the epidermis thickened over a small area, which is surmounted by a minute crust. There is no destructive process, only such a condition as might occur in the repair of an ordinary scratch. The papule excised nine days after inoculation shows a loss of epidermis over a minute area which is covered by a very delicate crust. (Such lesions were later found quite frequently in instances where the animal's belly was shaved, and were regarded as minute abrasions from scratching.)

Rabbit No. 2 — The eyes were anesthetized with two per cent cocaine solution and the exudate from the human skin lesions rubbed into shallow incisions made upon the cornea. The corneas appeared smooth after twenty-four hours and, as no process could be detected in the daily observations for a week following the inoculation, the animal was not killed.

Rabbit No. 3. — Both corneas were inoculated with the same exudate and in a similar manner as in Rabbit No. 2. The corneas in this case healed rapidly and no process developed subsequently. Animal not killed.

Rabbit No. 4. — Both corneas were inoculated with the crusts obtained from the inoculation of Monkey No. 1. The corneas became nearly smooth in from twenty-four to forty-eight hours. The animal was killed three days after inoculation. In sections of the cornea there appeared a slight defect in the epithelium at the point inoculated, but no pathological process beyond what might follow an ordinary injury was present.

A number of cases, developing subsequently among the prisoners were seen at what was evidently an earlier stage in the development of the eruption. These presented full hemispherical or oval vesicles which were easily ruptured and which contained clear fluid. Certain other cases seen with these presented lesions with a central crust surrounded by a vesicular zone containing either cloudy fluid, or whitish semi-fluid pus. Exudate was collected from both sorts of lesions and used to inoculate two monkeys.

Monkey No. 5. — The belly was shaved and the clear vesicle contents mentioned in the text was rubbed gently into superficial incisions made at intervals upon this area of the skin. Another portion of the clear fluid was blown into the nostril of the monkey. This animal developed no skin lesion while under observation for several weeks.

Monkey No. 6. — The skin of the belly was inoculated in a similar manner with the semi-fluid pus from the cases above mentioned. No lesions developed from this inoculation.

The negative character of these inoculations indicates clearly that the disease is distinct from smallpox. The result was the same whether the pus or the clear fluid was used. The virus of smallpox, even when collected from the mildest cases, produces a typical process whether inoculated on the skin of the monkey or the cornea of the rabbit. Furthermore, the clinical aspect of the disease, now that new cases were appearing, made the diagnosis of varicella, already indicated by the inoculation results, certain.

Before passing on to the description of the varicella lesions it may be well in this connection to give the results of some further experimental inoculations made at a later date. The fluid contents of vesicles was collected from three typical cases of varicella. In two cases the vesicles were perfectly clear, in the other case many vesicles were slightly clouded. The fluid collected was used to inoculate a series of seven rabbits. The mode of inoculation was first to anesthetize the eye with a few drops of two per cent cocaine solution so that it could be pressed gently from its socket and kept immobile while inoculating. In most instances two or three shallow parallel incisions were made across each cornea just

deep enough to penetrate the epithelium. The fluid to be inoculated was then placed upon the surface and rubbed gently into the incisions. The rabbits were killed at twenty-four-hour intervals from one to six days, and the corneas fixed in Zenker's fluid. The seventh animal was allowed to live for an indefinite period.

Rabbit No. 37. — Both corneas were inoculated with cloudy fluid from vesicles of case No. 32. The animal was killed twenty-four hours after inoculation. The corneal surface was nearly smooth.

Rabbit No. 38. — Killed seventy-two hours after inoculation with vesicle fluid from case No. 32. The corneal surface appeared smooth and without defect in epithelium. There was no conjunctivitis.

Rabbit No. 39. — The corneas were inoculated with clear fluid from vesicles of cases No. 34 and 35. Killed forty-eight hours after inoculation. The right eye showed marked conjunctivitis, and there were several minute whitish spots in the surface of the cornea.

Rabbit No. 40. — The corneas were inoculated with clear lymph from cases No. 34 and 35. Killed four days after inoculation. Corneal surface nearly smooth.

Rabbit No. 41. — Corneas inoculated with clear lymph from cases No. 34 and 35. Killed five days after inoculation. Corneas nearly smooth.

Rabbit No. 42. — Corneas inoculated with clear fluid from cases No. 34 and 35. Killed six days afterwards. Corneal surface smooth. No inflammation.

Rabbit No. 43. — Corneas inoculated with clear vesicle fluid from cases No. 34 and 35. This animal was not killed. The incisions of the corneal surface healed rapidly after the manner of aseptic wounds, and no apparent process developed during the fortnight which the animal was kept under observation.

In the histological study of the corneas of this series of rabbits nothing was revealed, with the exception of one case, beyond the usual process of repair which follows an uninfected wound. In Rabbit No. 39 one cornea showed a marked inflammatory process. Microscopically there were collections of bacilli growing in the depth of the incision and below the epithelium. The epithelium was thin and many cells were degenerated. The adjacent corneal tissue was infiltrated with leucocytes.

In no case did the gross or the histological appearances of the inoculated cornea suggest a process beyond that of repair, except one case in which there was considerable inflammation. In the latter case the inflammatory changes were evidently caused by masses of bacteria growing in the tissue of the cornea.

Grouping this latter series of inoculations with those previously done, there were in all nine rabbits inoculated upon the cornea with the vesicle contents of varicella. Of these five were inoculated with the clear fluid exudate, two with the clouded fluid, and two with the semi-fluid pus; the latter material came from cases which, although at first considered doubtful, were unquestionably varicella, as proved by further observations. In no case was the inoculation followed by any specific process. Three monkeys were inoculated upon the skin, one with clear fluid, and two with semi-fluid pus from varicella lesions; all gave negative results. Thus, up to the present time, it has not been possible to produce any form of the disease in animals by the inoculation of vesicle contents of varicella. Although the data furnished by the inoculation of three monkeys is meager, yet the results stand in marked contrast to those obtained through the inoculation of monkeys with variola virus.

Further study was directed to the pathological process concerned in the varicella eruption. As previously stated a study was made of thirty-eight cases, and attention was paid to the development of the eruption and the general condition of the patient. A more critical study with daily observations was made of eleven of the more favorable cases. These were selected because they were seen soon after the first appearance of the eruption and were uncomplicated by the appearance of skin lesions other than varicella.

Concerning the clinical features of the disease there was nothing presented more than may be found in the text-book descriptions. The cases were usually discovered within twenty-four hours after the appearance of the eruption. In some of the severe cases there was considerable fever and constitutional disturbance, but in the majority there was no complaint of feeling ill. All degrees of severity were represented, from mild cases in which there were not over a dozen lesions in the entire course of the disease to severe cases in which the lesions were thickly sprinkled over the entire surface of the body. Lesions were present in either the palms of the hands or the soles of the feet in twenty-seven

of the thirty-eight cases. The following case is given in order to convey an idea of the character of the disease:

Case No. 8. — Adult male Filipino seen on June 2. Eruption appeared within last twenty-four hours. Feels rather ill. He presents two good scars from former vaccination. An eruption scattered over trunk, arms, and legs, with only three lesions on face, consists of vesicles and small red macules. The vesicles are oval or hemispherical, superficial, tense, contain clear fluid, and are situated upon a reddened area of skin. The larger vesicles measure about five millimeters, the smaller ones are barely discernible. They are easily ruptured. The macules consist of small reddened areas of the skin, in some cases slightly elevated but barely palpable. In some such areas there is a minute central vesicle, in others the center is a rough, slightly granular elevation. Certain lesions were marked for future observation.

June 3. Several of the vesicles observed yesterday are now slightly clouded. Many macules have developed vesicles and certain vesicles have increased in size. Lesions numerous on back.

June 4. A great number of the vesicles have become clouded and are no longer tense. Few new lesions have developed except a number on the hands. Soft palate presents three grayish erosions. Several lesions on the face have been ruptured. One present in the scalp.

Beneath a small collodion dressing on the chest is a group of clear vesicles following the outline of the collodion.

June 6. Many of the vesicles have dried up and are represented by thin crusts. Others persist as flaccid vesicles containing cloudy whitish fluids. No new lesions have appeared since June 4. One lesion on trunk presents a depressed thin central crust and an elevated pustular edge.

June 7. Only three unruptured vesicles left on body. Many have disappeared or are represented by pale spots in the skin. In some cases, where the remains of the vesicle have been removed, there is a small round red area representing a defect in the epidermis. Around this is a pale translucent zone, representing the new epithelium growing in to replace that destroyed in the lesion.

June 8. Healing has proceeded still further. Many of the crusts have been removed leaving white rounded areas.

Lesions were excised from the eleven cases at various stages from the earliest reddening of the skin through their entire development until healing was well advanced. The technic of incision is as follows: A fairly large-sized scalpel, which must necessarily be sharp, is all that is absolutely necessary, although a pair of mouse-tooth forceps can be used to advantage in certain instances. The fixing fluid

should be already prepared and at hand. The skin is first washed with alcohol and sponged lightly with a piece of gauze or absorbent cotton to render it clean. The skin adjoining the lesion is pinched up between the thumb and forefinger so that the lesion is situated at the summit of the elevation. By holding the knife nearly flat with the surface of the skin, one cuts with a sawing motion beneath the lesion which, when removed, should be placed immediately in the fixing fluid. In certain cases, when the skin about the lesion is not sufficiently loose to be pinched up by the fingers, elevation may be accomplished by seizing the skin near the lesion with the mouse-tooth forceps and then excising. It is essential to work with a very sharp knife, otherwise the lesion is liable to become mutilated and the patient caused unnecessary pain. I would recommend this method for general purposes of diagnosis of superficial skin lesions, for the following reasons: It causes a very superficial injury amounting to scarcely more than a slight abrasion, which under ordinary conditions heals rapidly. By the thinness of the slice of tissue almost perfect fixation is obtained. By this method, in which the lesion itself is neither seized with forceps nor manipulated in any way, the topographical relations are undisturbed. The only objection to the method is that it cannot be done without a certain degree of pain, for the injection of a solution of cocaine so distends the tissue that it may be impossible to acquire any conception of the original process present. The method sometimes used of freezing the skin before excision is very objectionable. The blood is destroyed and the tissue cells are so altered that the subsequent staining is affected.

The tissue was generally fixed in Zenker's fluid. Lesions were excised twelve hours, one, two, three, four, five, six, and seven days after the eruption was first noted by the patient. On account of the appearance of successive crops of vesicles it was often possible to obtain early lesions late in the disease, so that the time interval, dated from the first appearance of the eruption, forms no index as to the stage of the process present. In order to acquire a more accurate knowledge of

the development of the lesions certain ones were ringed with indelible ink at their first appearance, and the subsequent changes noted.

The following description of the process is based on a study of a series of lesions of the type which appears most constant, beginning with the earliest stage found and taking up successive stages until repair is well advanced. In such a series of lesions the process is found to consist of changes occurring simultaneously in both epidermis and corium. However, for purposes of description, first the changes affecting the epidermis will be taken up, and subsequently the changes affecting the corium. The same order will be followed in the description of the later changes which result in the repair of the skin lesions. Following this the more unusual forms of the lesion will be described separately.

The earliest lesions are obtained by the excision of red spots which precede the formation of vesicles. The first change in the epithelial cells consists of a swelling of both the cytoplasm and the nuclei so that the epidermis is considerably thickened over a small area (Plate VII., Fig. 1). The process in the epidermis is often not confined to a single center. It is common to find both multiple distinct centers separated by normal epithelium, and multiple centers which merge into one another forming a conglomerate lesion (Plate VII., Fig. 2). With the swelling of the cells the cytoplasm stains more faintly and becomes distinctly reticulated. The nucleus is also distended so that it appears large and hollow. These nuclei almost invariably contain one or several masses of eosin-staining material. The nuclear network and the chromatin masses are retracted away from this material so that it appears to occupy a clear space within the nucleus. There are constantly present, in addition to the nuclear inclusions, small deeply-staining, rounded masses, situated on the outer surface of the nuclear membrane, in the cytoplasm, or between the epithelial cells. A discussion of the cell-inclusions associated with this process will be taken up in a subsequent paragraph.

Foreign cells occur here and there in the affected areas of

the epidermis. From the study of the changes in the underlying corium, it seems probable that these are cells of endothelial origin which have migrated into the epidermis. In many instances these extraneous cells are found in portions of the epidermis where there are but few cells deviating from the normal type. Thus very minute lesions of the epidermis are found in which no more than three or four cells show marked change (Plate X., Fig. 21). Some of the cells which have wandered into the epidermis are in a state of good preservation, and these often contain eosin-staining nuclear inclusions similar to those which occur in the epidermal cells. Others present various degrees of degeneration and many have become disintegrated. The small masses resulting from their disintegration may consist of either nuclear or cytoplasmic material, or both, and are readily distinguished.

The cell changes in the epidermis following the initial swelling are progressive. Two types may be distinguished. In that termed by Unna "reticulating colliquation," either the interior of the cell liquefies or a space is formed within it, while the periphery persists as a dense membrane. Within such hollow cells the nuclei are often collapsed, a condition possibly produced by changes in the surrounding cell fluid. This form of degeneration is not so prominent in the varicella lesion as that next to be described.

The term "ballooning degeneration" is given by Unna to certain changes resulting in the formation of large multinucleated cells with soft plastic cytoplasm. The first change is an increase in the size of the cell while the nucleus becomes large and vesicular. The nucleus then divides by direct division, and this process is rapidly repeated until a large number of nuclei are produced within the cell. The cytoplasm is basophilic, and in it a distinct reticulum appears. The limiting membrane and protoplasmic bridges of the cell become less distinct, and after a time disappear. Such cells attain enormous dimensions. The nuclei are usually grouped in the center of the cell, often so closely packed together that the opposing surfaces are flattened one against the other. These nuclei appear as hollow sacs with one or several masses

of chromatin peripherally situated, and with an eosin-staining inclusion in the interior of each nucleus. There is scarcely any indication of a nuclear network. In the presence of such a degree of amitotic division, it is nevertheless difficult to find nuclei caught in the act of division. The chromatin in some instances projects as a septum nearly across the nucleus. The dividing split passes directly through this projecting chromatin, so that an approximately equal portion goes to each of the resulting nuclei. Although this would seem, from the common occurrence of nuclei flattened against one another, to be the common form of direct division, yet it cannot be considered to occur constantly in this process. Certain nuclei are dumb-bell shaped, and the two portions are joined by a very thin filament. Some are lobulated and are apparently in the process of constricting off small portions of their substance, which form accessory nuclei of varying size. This latter form of nuclear division is seen in a greater degree in the migrating cells and in the endothelium lining the lymphatics of the corium than in the epithelium.

With the progressive cell changes in the epidermis, exudation becomes an important factor in the typical process. Fluid finds its way into the degenerated epithelium and separates the degenerating cells so as to form spaces filled with fluid. In this manner the formation of the vesicle is begun. That the exudate sometimes breaks into hollow cells is proved by the occasional presence of free nuclei in the smaller chambers. The exudate may at this early stage appear perfectly clear, or it may contain a small amount of fibrin either in the form of a reticulum or of a granular precipitate.

The continuous escape of the exudate into the epidermis rapidly dilates the smaller chambers into a large vesicle. In case the lesion has developed from multiple centers, the various chambers are separated by septa and trabeculæ which are prone to rupture on account of the rapidity of exudation. The chamber begins to form in the middle layer of the epidermis so that a portion of the stratum spinosum is lifted up with the horny layer to form the roof, while the

floor of the vesicle is at this stage wholly or partially covered with epithelial cells.

At the sides of the vesicle and in the trabeculæ the cells become elongated and stretched through the pressure of the exudate. With the cells thus stretched the epithelial fibers of Herxheimer become prominent. It seems evident that there is an actual production of fibers coincident with the swelling and stretching of the cells. They appear as straight fibers, with tapering ends, extending lengthwise over the surface of the epithelial cells. In some cases the cells become torn from their original attachment and the fibers then released take on a spiral or crinkly form (Plate VIII., Fig. 10). They vary much in thickness, and where cells lie free in the vesicle contents they often extend directly into the cell and terminate against the nucleus.

Lying free in the vesicle contents or in groups over the floor of the vesicle and along the trabeculæ are epithelial cells showing various forms and stages of degeneration, prominent among which are the large multinucleated cells resulting from the so-called "ballooning degeneration." These cells now present further degenerative changes. The cytoplasm is often condensed into a membrane about the nucleus or the group of nuclei. External to this membrane the cytoplasm is very soft in appearance, being composed of a delicate reticulum. In this delicate portion deeply-stained granules or masses appear. On the surface of such cells there are sometimes globular or elongated masses staining faint blue, which radiate from the cell like the petals of a flower. In many cases the uniformity of these bodies and their arrangement is suggestive of some sort of an organism. After studying a number of cells of this type the observer is convinced that the masses are pseudopodia-like projections from the soft protoplasm or portions of it which have been constricted off. In some cells the so-called "acidophilic" or "fibrinoid" degeneration has become marked, and the entire protoplasm appears hyaline and deeply stained with eosin. The hyaline transformation makes its first appearance about the nuclei either in the form of a dense membrane or as irregular masses.

The base of the vesicle in which there is an active exudation is almost invariably concave. The papillæ are either totally obliterated or distorted through lateral distension. The epithelium in many cases persists between these papillæ for some time after it has been destroyed over the more exposed portions. The vesicles are in some instances multilocular, but the average vesicle is unilocular and stands in marked contrast to the type of lesion found in smallpox. The common involvement of hair follicles and sweat ducts in the typical rounded vesicles indicates clearly that these structures acting alone do not produce umbilication.

After the vesicles have become fully formed other changes occur. To the naked eye the vesicles are flaccid and no longer tense, and the fluid is clouded. This condition may be seen three or four days after the first appearance of the eruption, or it may be present even earlier. The evaporation and absorption are now more rapid than the outpouring of the exudate. The fluid within the vesicle contains more fibrin than in the early stages, and phagocytes which are attracted by the dead epithelial cells are beginning to appear. The roof of the vesicle, the trabeculæ, and the cells of its lateral wall now take a diffuse stain, and are apparently dead. The trabeculæ are ruptured in almost every instance, and their remains are to be found projecting downward from the roof of the vesicle. The former pressure of exudate has in many instances broken peripherally beyond the boundary of the original lesion so that irregular spaces are found. The large rounded epithelial cells lying loose on the floor of the vesicle show various degrees of hyaline changes. Some appear dense and hyaline, others are stained but faintly. The nucleus is either no longer apparent or is represented by irregular deeply-stained masses. Probably all the epidermis involved in the lesion is eventually destroyed.

In some instances a portion of the epithelium, which shows none of the cell changes that are characteristic of the process, is invaded by numbers of phagocytic cells. It is possible that such infiltration of a small focus of the epidermis represents the site of a minute process in which the initial cell changes

have been obscured in the infiltration which has followed. The phagocytic cells invade the normal epidermis, and are found in varying number far from any well-defined vesicle. Cause for their presence is not to be found in any injury to the epidermis.

In all the early lesions a pathological process is constantly found in the corium. The first change noted is the presence of eosin-staining inclusions situated usually in the nucleus, more rarely in the cytoplasm of cells. In what may be considered a very early stage in the process the eosin-staining inclusions are limited to the endothelial cells lining the small blood vessels. Later, when the process is well advanced, similar inclusions occur in a large proportion of the cells in the corium underlying the vesicle, and in almost every type of cell here represented. These inclusions are associated with cell changes similar to those already described in the affected epidermis. The nuclei become distended and tend to divide by amitosis without subsequent division of the cytoplasm. Thus early changes of this type are found constantly in the corium as well as in the epidermis of the varicella lesion. It seems probable that the changes in the corium antedate those in the epidermis, for they are always present and often well marked in the earliest and most minute lesions.

Further changes occur in the corium, appearing at first chiefly in and around the blood vessels. The process is more advanced in the neighborhood of the epidermal foci, but slight changes occur over much larger areas. The endothelial cells lining the small blood vessels present various degrees of proliferation as shown by the presence of numerous mitotic figures. Immediately around the vessels are collections of cells of which the most numerous are cells having a large vesicular nucleus and more or less basophilic cytoplasm. It seems probable that these cells are of endothelial origin, arising from endothelial cells in the lymph spaces and vessels adjoining the blood vessels. In favor of this view is the fact that numerous mitotic figures occur in the cells lining these lymph vessels. Later in the process these

endothelial cells become phagocytic. Mingled with these cells are a few lymphoid cells, an occasional eosinophile, but the polymorphonuclear leucocyte is not represented.

In addition to the early cell changes already described, more advanced degeneration is found in cells in the vicinity of the affected vessels. The degeneration is not general; only scattered cells are affected. In some instances an entire cell appears hyaline and stains intensely. In other cells a form of nuclear fragmentation has taken place, and the chromatin is scattered throughout the cell in the form of minute deeply-stained globules. Portions of disintegrating cells are found occasionally. The destruction is often so far advanced that it is impossible to determine, in single instances, the type of cell affected, but it seems certain that the cells previously described as of probable endothelial origin are frequently destroyed. Changes in the corium, such as those already described, are found in all early lesions.

In lesions taken at a later stage, during the formation of the vesicle in the epidermis, a more extensive process is found in the corium. The endothelial cells, although still most numerous about the blood vessels, are not confined to this locality. Large numbers are found scattered through the corium beneath the affected epidermis. The number of these cells migrating in the tissue varies considerably, however, in different lesions.

It is not uncommon to find the capillaries and small vessels dilated just beneath the vesicle. The lymphatics also are dilated in some instances but, with the exception of those which occur immediately about the blood vessels of the lesion, there seems to be no noteworthy change affecting them.

In some lesions a great proportion of the cells in the corium below the vesicle present those peculiar changes characterized by the presence of eosin-staining nuclear inclusions and direct nuclear division. The cells are transformed to such a degree that it is often impossible to decide to what type certain cells belong, but it is evident that nearly all varieties of cells are affected, including endothelial cells,

both in situ upon the vessel wall and free in the tissue, connective tissue cells, pigment cells, and eosinophiles. The nuclei become swollen, irregular, and lobulated, and segment irregularly into smaller nuclei often of unequal size. The cytoplasm is increased to such an extent that cells of relatively enormous size are produced. When such large cells are of connective tissue origin the cytoplasm presents irregular branching processes. Occluded blood vessels were found in several instances. The occlusion was due to the presence of large multinucleated cells within the vessels. In another instance a group of large multinucleated cells extended through sixteen sections (eighty micromillimeters) of the tissue in close relation to a blood vessel. It would seem that large multinucleated cells occasionally develop from the endothelium of the blood vessels and lymphatics involved in the lesion.

Later in the process phagocytosis becomes a prominent feature. The endothelial cells are found in varying numbers in the cavity of the vesicle where they are engaged in devouring the remains of epithelial cells. That portion of the corium which forms the floor of the vesicle is infiltrated with these phagocytic endothelial cells. Phagocytosis is found in a greater or less degree throughout the corium of the lesion. At this stage small numbers of polymorphonuclear leucocytes are present in the corium. On the other hand, if bacteria have gained entrance, polymorphonuclear leucocytes are found infiltrating the tissues in great numbers. Their appearance seems to be of the nature of a secondary phenomenon rather than an essential part of the process.

In the majority of lesions the active process ends soon after the destruction of the involved portion of the epidermis is completed. Many of the resulting necrotic epithelial cells appear either as dense hyaline or as faintly stained masses both free in the exudate and along the floor of the vesicle.

Phagocytic cells are present in varying numbers, but there is usually a large number present in the cavity of vesicles of four days duration. In the uncomplicated process polynuclear leucocytes are relatively rare, even in the older vesicles.

When the destructive process is at an end, the demarcation between the living and the necrotic epithelium becomes distinct. The former grows inward over the floor of the vesicle following the surface of the papillæ. This rapidly-growing epidermis presents the same characteristics as that growing over any defect. The absence of pigment in the newly-formed epidermis accounts for the whiteness of recently healed varicella lesions in Filipinos. The vesicle may persist as such until the surface beneath is completely healed, or it may be ruptured and form a thin crust on drying.

In the corium the migrating cells become gradually reduced in numbers, the infiltration about the blood vessels is diminished, and with the repair of the defect in the epidermis the underlying tissue also returns to its normal state.

In addition to the typical lesions upon which the description of the process has been based, certain other atypical lesions occur.

Vesicle within vesicle. — In a certain percentage of the lesions there is a secondary extension of the vesicle. After the vesicle is apparently well developed it still remains tense and, when seen on the following day, an increase in size is noted, and the outline of the former vesicle is seen within the larger lesion. The original vesicle is clouded and opaque while the newly-formed vesicle is clear. This condition is brought about by the leakage of fluid from the cavity of the original vesicle into the horny layer which it splits (Plate IX., Fig. 15). It seems probable that the larger blebs and bullæ, sometimes met with in these cases, are formed in this way as they appear to be very superficial.

Dry or abortive lesions. — In a small number of lesions there appears a marked localized degeneration of the epithelial cells unaccompanied by any appreciable exudation. These lesions, appearing as reddened slightly elevated spots in the gross, will show microscopically a portion of the epidermis wholly necrotic, the cells of which are disassociated, stain red, and are hyaline in appearance like those in the advanced vesicle. Reticulating colliquation is not represented. Such lesions are simply lacking in fluid exudation.

(Plate IX., Fig. 16). This condition is difficult to explain especially when cases occur in which a majority of the lesions develop in this way. A case illustrating this condition is as follows:

Case No. 28. — June 8, 1904. A Filipino young adult, having two old vaccination scars. The eruption has appeared within the last twenty-four hours, and is scanty. It is papular on the face, and mixed with acne. Several clear vesicles, five to six millimeters in diameter, in groin, few papules on legs. A vesicle, five millimeters, on anterior thorax; one, two millimeters, back of the shoulder; and a few small vesicles on arms and forearms.

June 9, 1904. Eruption has increased and is scattered over the entire body. It consists of small reddened spots, the majority of which have a slightly elevated granular surface. None on hands or wrists. Face rough and papular. Mouth negative.

June 10, 1904 The eruption is now profuse and simulates an early smallpox eruption. It consists of small red papules, two to four millimeters across, with granular tops. Some papules have at their summit a tiny vesicle in the center of a slightly roughened surface. A few clear hemispherical vesicles have developed. Eruption is profuse on face and trunk, scattered over arms and legs, none on palms.

June 11, 1904. Very few papules have become vesicular. Many lesions have faded out and practically disappeared. Following this date no new vesicles appeared and those present healed rapidly.

Lesions in the hair follicles. — It is common for hair follicles to be involved in the vesicle of varicella, but the process is at times so limited that it does not reach the surface of the skin. This occurred in two lesions excised. In one was an early process affecting the sebaceous gland and hair sheath (Plate VIII., Fig. 8). In this lesion were all the forms of cell degeneration already described. There was but slight infiltration with wandering endothelial cells and no vesicle formation. In another lesion similarly situated the sebaceous gland and the lower portion of the hair sheath were infiltrated with endothelial cells, and there was a small chamber containing fluid. In places the cell degeneration could be made out to such an extent as to identify the process as varicella.

Lesions about the ducts of sweat glands. — These ducts are involved in a great many lesions. They may be seen at

points along the base of the vesicle (Plate IX., Fig. 15) or portions may persist within the chamber of the vesicle. Some of the minute foci are found about the epidermal portion of these ducts (Plate VII., Fig. 4). In such instances the cells lining the duct are unaffected while the process is confined to the epidermis which surrounds it. In no case was the characteristic initial change of the process found in these ducts.

Vesicle infected by bacteria. — A certain number of lesions do not heal immediately after the full development of the vesicles. The contents become white and opaque, and the vesicle is topped with a central crust. Vesicles presenting such an appearance have probably been ruptured, so that bacteria have gained access, although it is possible that bacterial infection may in some instances occur without the vesicle being ruptured. Such lesions extend slowly, the central crust becomes larger and surrounded by a pustular ring. The depression of the central crust as compared with the elevated pustular periphery furnishes a form of umbilication. It is plain that this lesion is secondary to varicella and has nothing to do with the active process. The umbilication is therefore of a false variety. On account of the inadvisability of excising such lesions, only one small lesion of this character was obtained. In this the pustule contents was composed almost wholly of polymorphonuclear leucocytes. The corium forming the base of the pustule was infiltrated throughout with these leucocytes, and there was considerable destruction of tissue in the papillary layer. It seems probable that the permanent scars, which are not an uncommon sequence of varicella, result from lesions of this type. In the milder uncomplicated lesions, in which the papillary layer is not perceptibly damaged, the regeneration of the destroyed epithelium constitutes perfect repair and no scar is produced.

The earliest changes in the varicella process are associated with cytoplasmic and nuclear inclusions which are found in epidermal cells, endothelial cells in situ within the blood vessels and lymphatics, as well as those which are found

migrating in the tissue, connective tissue cells, pigment cells, and eosinophiles.

The nuclear inclusions appear at first as minute eosin-staining granules about one micromillimeter in diameter, situated in the nuclear sap. The nucleus may contain one or several. A clear space is formed about each nuclear inclusion and the nuclear network recedes from it. The inclusions rapidly increase in size and stain more intensely. They are most often rounded, but may be elongated or irregular. In some instances the nucleus is affected only about the inclusion, at which point it is greatly distended, while the remainder appears normal (Plate IX., Fig. 18). Very irregular and lobulated nuclei are thus produced in the cells of the corium. In the epithelial cells the whole nucleus becomes distended, the chromatin and nuclear network retracts to the periphery, leaving the interior about the inclusion clear. Direct nuclear division follows these changes, and each of the resulting nuclei usually contains a characteristic inclusion. These inclusions may attain a diameter of six microns when rounded, and nine microns when stretched out in elongated form. Some appear dense and homogeneous, while others are granular in character. Indefinite deeply-stained points are occasionally to be made out in them. In some instances minute granules of similar substance are grouped about a large inclusion. In one case in which the knife had passed through a sebaceous gland in excising the lesion, the nuclei were ruptured and the inclusions here showed characteristics not apparent elsewhere (Plate VIII., Fig. 12 and Plate X., Fig. 25). They were evidently composed of a plastic substance, as they had a sharp though irregular outline with rounded projections suggestive of a flowing motion. They consist of a sharply-contoured body delicately reticulated in structure. Certain ones showed a central deep-staining granule. A certain number of nuclear inclusions are found which either are attached to the nuclear membrane or are protruding through it. They show a marked constriction where they pass through the membrane. A large proportion of these inclusions present the form of an irregular reticulated mass

without definite contour. Although these inclusions take the red in preference to the nuclear stain, they stain deeply by other methods so that they would not readily be distinguished from chromatin.

The cytoplasmic inclusions stain a dense purple by the eosin and methylene blue method, and measure from one to four micromillimeters in diameter. They are often found lying against the outer surface of the nuclear membrane, but may also occur between cells, free in the connective tissue, or in any position in the cytoplasm. They are constantly present in moderate numbers in the epidermis and in the cells of the corium of the early lesions, but are usually less numerous than the nuclear inclusions. They always possess a sharp contour, are most often rounded, but are occasionally irregular, and have usually a deeply-stained central point. The occurrence of nuclear inclusions protruding through the nuclear membrane suggests a possible method of origin for the cytoplasmic bodies. They do not appear to be related to the fragments of disintegrated cells which are of common occurrence in the lesion.

It is difficult to reach a conclusion concerning the nature of these bodies. They are associated with the earliest changes and constitute one of the constant characteristics of the process. In a small portion of the inclusions there is some evidence of internal structure, but this is so indefinite that it carries but little weight as to their nature. A large proportion of the nuclear inclusions are so structureless and irregular that it is difficult to consider them as living organisms. In spite of the great rapidity of the process it has not been found possible to demonstrate a multiplicative process in these bodies.

In the fresh preparation they appear homogeneous, and a large number of inclusions were watched for several hours under the microscope during which time they did not change in outline. Furthermore, eosin-staining material is found within the nuclei in various other processes and in normal tissues. The writer is, however, familiar with no condition which presents inclusions closely simulating those found in varicella lesions.

As the earliest nuclear inclusions appear as red-staining granules in the nuclear sap, it is possible that they represent an increase of a substance, such as linin, present in normal nuclei. It seems to be certain that elements, readily overlooked in normal tissue, may become prominent in pathological conditions. Such an example is found in these same varicella lesions in which the epithelial fibers of Herxheimer, which are occasionally met with in normal epidermis, become increased to such an extent that they form one of the most striking features of the process (Plate VIII., Fig. 10). In this example, however, the elements in question can be traced in their transition from normal structure, and are always confined to the epidermis. The nuclear and cytoplasmic inclusions, on the other hand, present a constant morphology without transitional stages connecting them with normal structures, and are not confined to cells of any one tissue or type of tissue.

Another plausible hypothesis is that the inclusions represent the formation in a pathological condition of some substance not found in the normal nucleus. The reaction of the nucleus accompanying the presence of the inclusion is peculiar in that only that part about the inclusion is affected, while the remainder of the nuclear structure appears normal. If the inclusions are the result of a changed metabolism one would expect to find a more general change. A fact to be emphasized concerning the nature of these inclusions is that they are confined to small foci, even to individual cells in the epidermis and corium. There is not sufficient evidence at hand to interpret either the nuclear or cytoplasmic inclusions, and it seems preferable to suspend one's judgment until new facts are acquired bearing on this question. They are described because they form a constant and prominent feature in the varicella process.

The failure to demonstrate that the disease can be communicated by inoculation with the contents of the vesicles is against the idea that the infectious agent is contained in them. This evidence is not absolute and, moreover, a necessary corrollary of such experiments would be the excision and

study of the area inoculated. It might well be that a slight but characteristic lesion may be produced which would not be apparent on macroscopic examination, and which would not lead to the production of an exanthem. If we regarded the production of the entire disease as necessary to constitute infection, the infectiousness of smallpox in the inoculation of the calf or rabbit would be denied. On the other hand, the lesions in the disease are so cellular in character, and the cellular changes in their entirety so characteristic, that it is hardly conceivable that the process can be produced by anything other than some agent which enters into and affects the single epithelial cells.

That the infectious agent is disseminated by the blood is rendered probable by the common occurrence in early lesions of a process in the corium of wider distribution than is found in the epidermis, and by the occurrence of isolated lesions of blood vessels far from any epidermal involvement. It seems reasonably certain that the process in the corium antedates that in the epidermis.

Certain facts brought out in this investigation may be given a practical application in the matter of diagnosis. During every epidemic of smallpox there occur cases in which there is great difficulty in diagnosis. One needs but to scan the literature to acquire a conception of the number of these difficult cases. Stelwagon[14] quotes an analysis of thirty-eight cases of error in diagnosis of smallpox, of which seventeen turned out to be varicella. There has been some dispute in regard to the nature of a widespread epidemic reported from Trinidad, whether the cases were all varicella, or some a mild form of smallpox. There is no doubt that many cases are passed over without their true nature being recognized. One cannot depend absolutely on the gross appearances of the lesions, or on the distribution.

It seems a matter of considerable importance to be able to distinguish certain cases of smallpox resembling varicella, which although mild may give rise to severe variola in other individuals, from true varicella.

Certain methods concerning the diagnosis of smallpox

have already been suggested. The inoculation of monkeys, as suggested by Park,[11] is not always practicable in that monkeys are expensive and not always obtainable.

Howard[10] suggests in addition to animal inoculation the study of sections of excised skin. The diagnosis is based upon the character of the process and the presence of C. variolæ.

Thompson[17] has applied a rapid method for imbedding the excised lesions, by which he is enabled to obtain sections ready for study within three hours after excision.

The following methods are given since they may be found of value in the diagnosis of cases in which there is a question of smallpox or varicella:

1. The contents of early clear vesicles of such a case may be examined fresh under the microscope. The presence of large multinucleated cells is consistent with varicella and against smallpox. This test seems quite reliable and may be applied at the bedside.

2. The excision of a typical lesion for histological examination may be resorted to. In the smallpox lesion the presence of C. variolæ will furnish the diagnosis. In varicella the type of the vesicle, the ballooning degeneration, and the constant presence of the described intranuclear and cytoplasmic inclusions will render certain the diagnosis. As will be readily inferred, the simple presence of definite cytoplasmic or nuclear inclusions within the epithelial cells is not sufficient to establish the diagnosis of smallpox, for both nuclear and cytoplasmic inclusions are also found in varicella. If the presence of these bodies is to be taken into account in the diagnosis one must possess a certain degree of familiarity with them, in one or both of the diseases in question, in order to determine their specificity.

3. A slower but very reliable means of diagnosis is the inoculation of a rabbit's cornea with fluid from the lesions. With smallpox lymph a variolous keratitis is produced which is evident in the roughening of the corneal surface in from twenty-four to forty-eight hours after inoculation. The inoculation of varicella lymph gives no process.

SUMMARY.

The study of lesions excised from eleven cases of varicella in adult Filipinos shows that the initial change consists of the appearance of peculiar eosin-staining inclusions within the nuclei and cytoplasm of epithelial and various other cells. Direct division of nuclei without subsequent division of the cytoplasm is associated with these inclusions. Cells undergoing these changes often attain relatively enormous dimensions (the ballooning degeneration of Unna). This type of degeneration is most prominent in the affected areas of the epidermis, but occurs also in almost every type of cell in the corium. The epidermis presents also the reticulating type of degeneration, but only in a minor degree.

Following these cell changes the typical varicella process consists of a rapid destruction of small areas of the epidermis, associated with exudation and vesicle formation. The exudate penetrates the injured area of the epidermis forcing the degenerating cells apart and forming spaces which tend to coalesce to form a single chambered vesicle. Exudative cells, probably of endothelial origin, are occasionally met with in the epidermis very early in the process. Collections of similar cells are found about the blood vessels of the underlying corium. Later in the process they are present in the corium in large numbers and exhibit ameboid activity and phagocytosis. The exudate filling the vesicle is at first clear and contains only fibrin and a few degenerating or necrotic epithelial cells, but later large numbers of phagocytic endothelial cells are found in it. The active destructive process is followed at once by repair. The epidermis grows in, closing the defect, and the corium returns to its normal condition.

From the wider distribution of the process in the corium than in the epidermis, and from the occasional occurrence of a process in a blood vessel far removed from any epithelial focus, it seems probable that the first change in the corium antedates that in the epidermis.

The development of the vesicle is attended by the formation, about the epithelial cells, of numerous fibers which

agree very closely with the fibers of Herxheimer found in the normal epidermis. It is apparent, however, that there is actually an increased production of these fibers in epidermis stretched through the pressure of the exudate.

Several varieties of atypical lesions occur. The exudate may break into the wall of the vesicle and separate the horny layer so that a secondary vesicle is formed which includes the original, or lesions may occur practically without exudation. Some lesions are confined to the depths of hair follicles without the surface epithelium being affected. A certain number of lesions are infected by bacteria, as the result of which they become pustular. In such lesions there is more or less destruction of the papillary layer, which accounts for the scars frequently following an attack of varicella.

Specific nuclear and cytoplasmic inclusions are found in all varicella lesions, and their appearance constitutes the earliest change observed in the tissues. They occur in epidermal cells and in various cells of the corium. The nuclear inclusions stain red or purplish by the eosin, methylene blue method, and vary in size from one to six microns in diameter. The cytoplasmic inclusions stain a deep purple, and a central granule is apparent in many. They are seldom found measuring over four microns. A conclusion has not been reached concerning the nature of these inclusions in varicella, and no important evidence has been found in favor of the hypothesis that they are parasitic organisms.

Inoculations of the cornea of the rabbit and the skin of the monkey with the contents of varicella vesicles have in all cases yielded negative results.

REFERENCES.

1. Buchmüller, A., 1886. Varicella, eine Krankheit sui generis. Mittheil. des Vereins der Aerzte Steiermarks. Ref. Virchow-Hirsch, Bd. 1886, 2, 44.

2 Czarkert, 1869. Zeit. d. Wundäzte Oesterr. Ref. Ziemsen's Encyclopedia, ii, Am. Edit.

3. Fleischmann, 1870. Die Stellung der Varicell zur Variola. Wiener Med. Woch., N. 51.

4. Fleischmann, 1871. Ueber Varicella und Varicellen-Impfungen. Arch. f. Derm. u. Syph., 1871, xiii, 497.

5. Freyer, M., 1892. Zur Frage der Identität von Varicellen und Pocken. Zeit. f. Hygiene, 1892, xii, 305.

6. von Genser, Th., 1903. Sind Varizellen eine ausschliessliche Kinderkrankheit? Wiener Med. Woch., 1903, iii, 124.

7. Heim, 1809. Horn's Archiv., 1809, x, 183. *Ib.*, 1825, 1. Ref. Ziemsen's Encyclopedia, ii, Am. Edit.

8. Hesse, 1829. Ueb. Varicellen, etc. Leipsig, 1829. Ref. Ziemsen's Encyclopedia, ii, Am. Edit.

9. d'Heilly, 1885. De la spécificité et de l'inoculabilité de la varicelle. Bull. et mem. Soc. Méd. d. hôp. de Paris, 1885, ii, 337.

10. Howard, W. T., 1905. The application of laboratory methods to the diagnosis of variola. J. Amer. Med. Assoc., 1905, xliv, 1095.

11. Park, W. H., 1902. A practical method of differentiating between variola and varicella by means of the inoculation of monkeys. Trans. Assoc. Am. Phys., 1902, xvii, 217.

12. Smith, J. L., 1896. A treatise on the medical and surgical diseases of infancy and childhood. Lea Bros., N.Y. and Phila., 1896.

13. Steiner, 1875. Zur Inokulation der Varicellen. Wiener Med. Woch., xxv, N. 16, 306.

14. Stelwagon, 1902. Diseases of the Skin, — Text-book.

15. Thomas, L., 1869. Ein Beitrag zur Kenntniss der Varicellen. Arch. f. Derm. u. Syph., 1869, iii, 329.

16. Thomas, L., 1875. Ziemsen's Encyclopedia, ii, Am. Edit.

17. Thompson, R. L., 1905. Laboratory diagnosis of variola. Bulletin of St. Louis University, 1905, i, 66.

18. Unna, P. G., 1894. Varicellen. Orth's Path. Anat., 1894, 2½, 634.

19. Unna, P. G., 1900. Histologischer Atlas zur Pathologie der Haut, 1900, Heft 4. Hamburg u. Leipzig.

20. Vetter, 1860. Impfung von Varicellenlymphe. Arch. der Heilkunde, 1860, i, 286.

21. Vetter, 1864. Ueber das Verhalten der Varicellen zu den Pocken. Virchow's Archiv., 1864, xxxi, 400.

DESCRIPTION OF PLATES.

PLATE VII.

FIG. 1. A minute early lesion. The epidermis is thickened over a small area, the result of the swelling of cells. The nuclei are large and vesicular and several contain the characteristic inclusions.

FIG. 2. An early varicella lesion showing multiple centers of degeneration in the epidermis. The ballooning type of degeneration is most prominent. Exudate has penetrated the epidermis separating the cells and forming small chambers.

FIG. 3. A minute varicella vesicle presenting a defect in the epidermis forming its floor. The exudate contains a small amount of fibrin. Considerable infiltration of the underlying corium.

FIG. 4. The process affecting the epidermis about the duct of a coil gland, which, however, is not involved. Three large multinuclear cells resulting from direct nuclear division are present in the degenerated portion of the epidermis.

FIG. 5. A minute lesion taken within twenty-four hours after the first appearance of the eruption. Exudate has penetrated the degenerated epidermis forming small spaces. This represents the beginning of vesicle formation.

FIG. 6. A typical unilocular vesicle taken twenty-four hours after the first appearance of the eruption. The fluid filling the chamber contains a small amount of fibrin and a few epithelial cells which have become loosened in the exudation. The epithelium persisting beneath this chamber presents various degrees of ballooning degeneration.

PLATE VIII.

FIG. 7. Vesicle, taken twenty-four hours after the first appearance of the eruption, with lense-shaped chamber, extending through the axis of which is a hair sheath. The fluid contents of this vesicle appeared perfectly clear macroscopically, but contains a small amount of fibrin. Slight infiltration about the vessels of the corium.

FIG. 8. A lesion located in the depths of a hair follicle and presenting several large multinuclear epithelial cells. The surface epithelium is not affected.

FIG. 9. Two characteristic cytoplasmic inclusions situated in the epidermis. x 1,000.

FIG. 10. Coarse fibers found in the epithelium forming the wall of the vesicle. x 1,000. Stained with phosphotungstic acid hematoxylin.

FIG. 11. Flaccid, slightly clouded vesicle of two days duration. Large amount of fibrin in exudate. Epidermis involved in lesion almost completely necrotic.

FIG. 12. A large nuclear inclusion found in a sebaceous gland. Some indication of internal structure. x 1,000.

FIG. 13. Vesicle of four days duration. Fluid contains large numbers of leucocytes and phagocytic cells. Epidermis is commencing to grow in at the sides.

FIG. 14. Corium in the vicinity of a varicella vesicle. The pigment cell to the left of the capillary contains a large number of nuclei probably resulting from a mitotic division.

PLATE IX.

FIG. 15. A vesicle of six days duration. A break has occurred in the lateral wall of the vesicle at some time during its development, allowing the fluid to penetrate and separate the horny layers. A coil gland duct extends down from the floor of the vesicle. Repair is well advanced and the epithelium is growing over the defect upon which the vesicle is situated.

FIG. 16. A minute "dry" lesion showing advanced degeneration of the epithelial cells without any appreciable fluid exudate.

FIG. 17. Multinuclear epithelial cells shown at lower magnification in Fig. 4. Outline irregular, without definite cell membrane, cytoplasm presents a loose reticulum, nuclei hollow, with chromatin situated near nuclear membrane, and usually containing the specific inclusions.

FIG. 18. Cells, probably endothelial in origin, situated in corium. They show localized swelling of the nucleus about the specific inclusions.

FIG. 19. An atypical vesicle, multilocular in type and showing a central crust. The base is infiltrated with leucocytes.

PLATE X.

(*Camera lucida drawings with Zeiss comp. oc. 6, obj. 2 mm., apert. 1.30.*)

FIG. 20. Tissue about a small blood vessel of the corium just beneath an early varicella vesicle. An endothelial cell in situ upon the vessel wall contains a small inclusion within its nucleus. Other nuclei in the tissue about the vessel contain the characteristic red-staining inclusions situated in clear spaces or vacuoles in the nucleus.

FIG. 21. Skin excised early in the disease. The epidermis of the Filipino contains a large amount of pigment. A single endothelial cell containing a nuclear inclusion is shown. Another endothelial cell found in another optical plane and also containing a nuclear inclusion is not shown. The epithelial cell above shows a characteristic nuclear inclusion. The study of serial sections failed to show any other similarly affected cells in this portion of the epidermis, so that it is to be regarded as a very minute lesion.

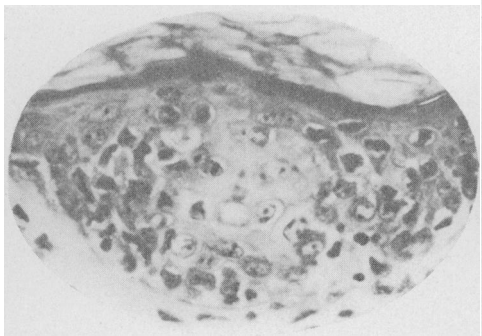
FIG. 22. Nuclear and cytoplasmic inclusions in the cells of both corium and epidermis.

FIG. 23. An epithelial cell possessing two nuclei, one of which appears hollow and contains a red-stained body. Just outside the nuclear membrane is a more intensely stained body.

FIG. 24. Epidermal cells in the floor of developing vesicle. Amitic nuclear division has here resulted in a large multinuclear cell containing inclusions in several of its nuclei, and also one extra nuclear inclusion. Other inclusions are present in adjacent cells. Some of these inclusions, especially the cytoplasmic, have deeply-stained central points.

FIG. 25. Cells from a sebaceous gland through which the knife passed in excision. Many of the nuclei containing inclusions have been ruptured. The inclusions are relatively large and show in certain instances a suggestion of reticular or vacuolar structure. The deeply-stained granules present in two may possibly be superimposed or include chromatin from the cell nucleus.

FIG. 26. An epidermal cell from a varicella vesicle showing the extension of a portion of the specific nuclear inclusion through the nuclear membrane.



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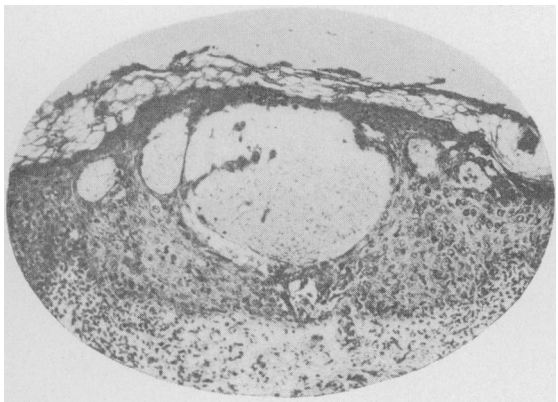

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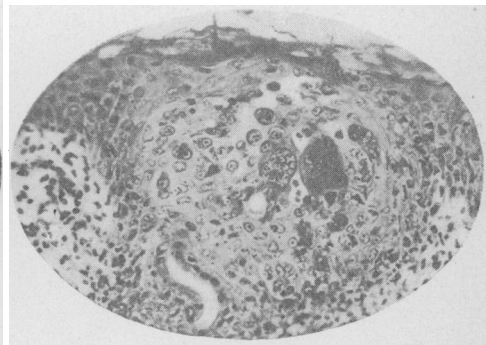

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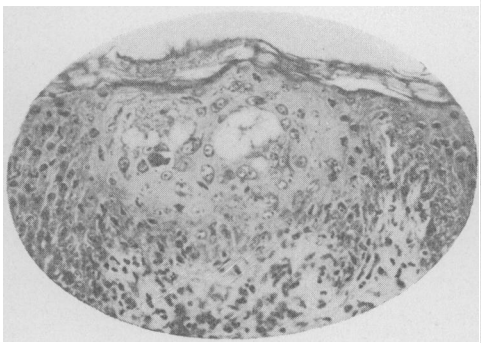

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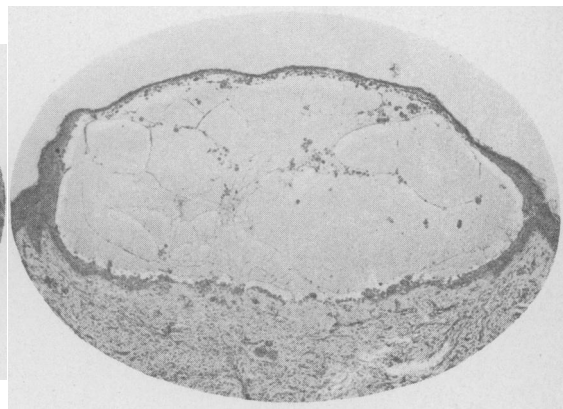

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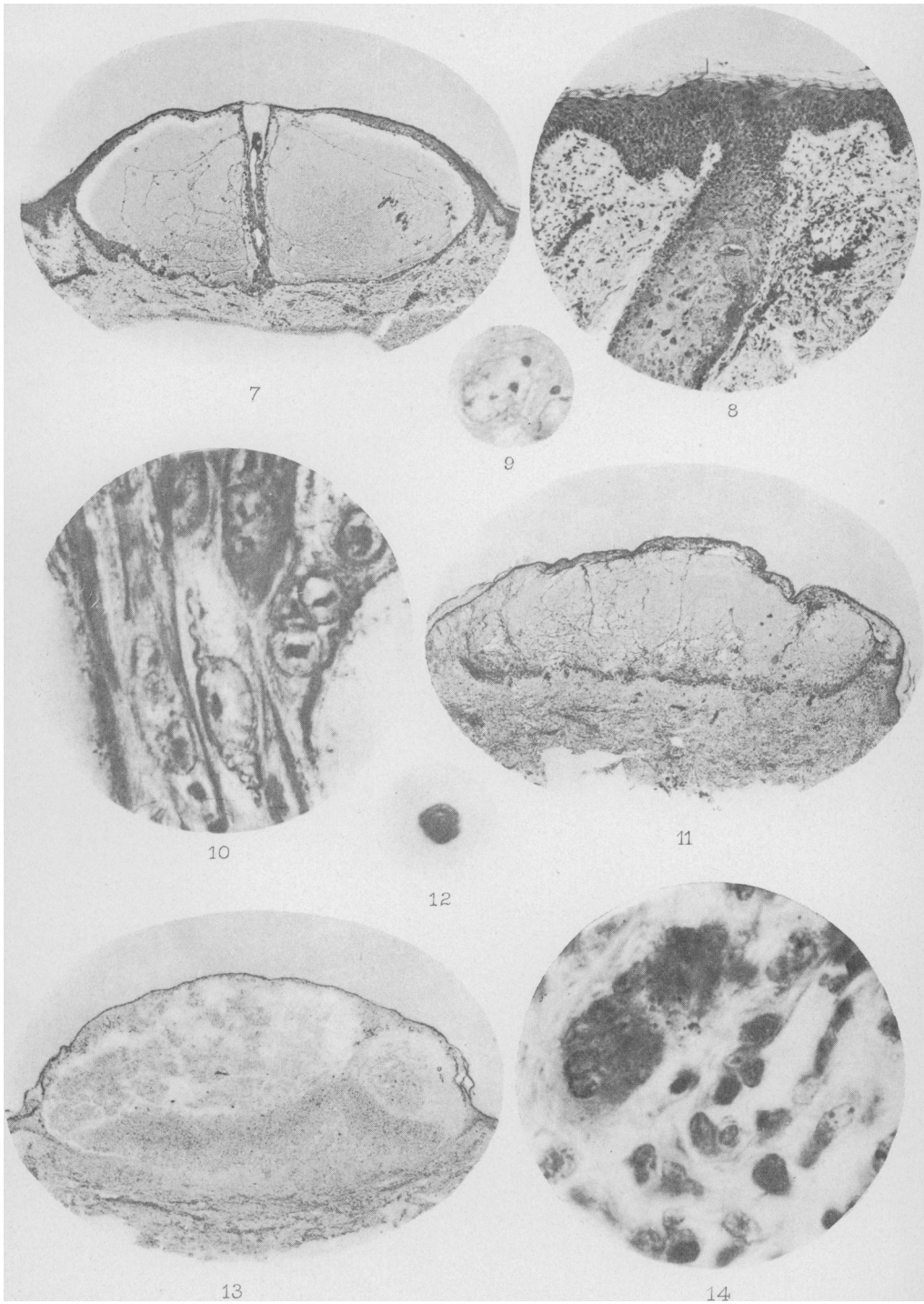
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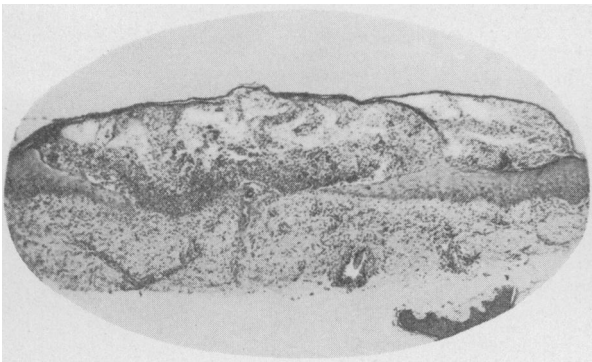

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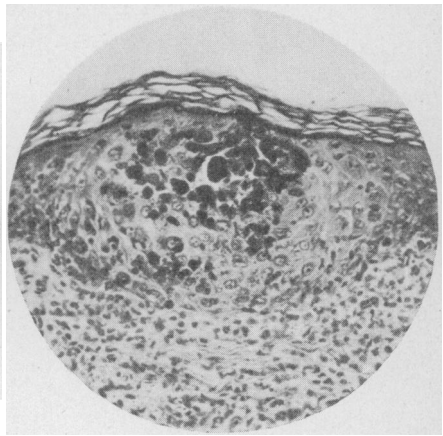

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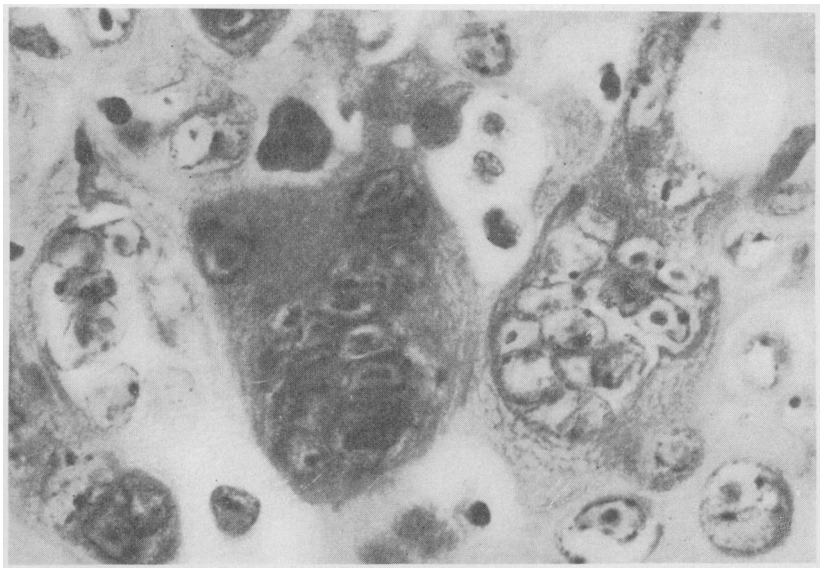

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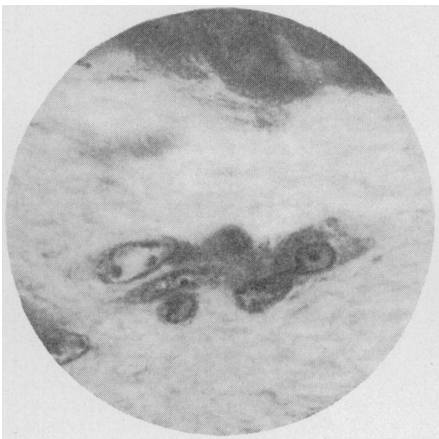

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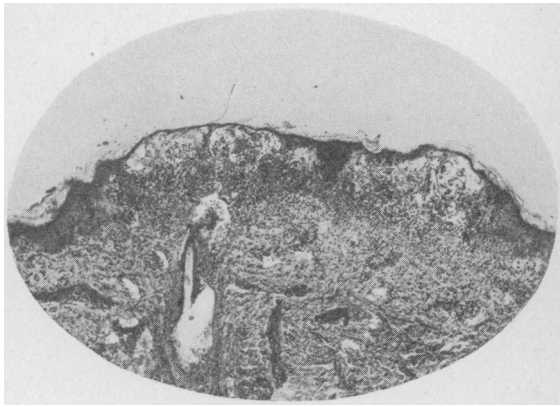

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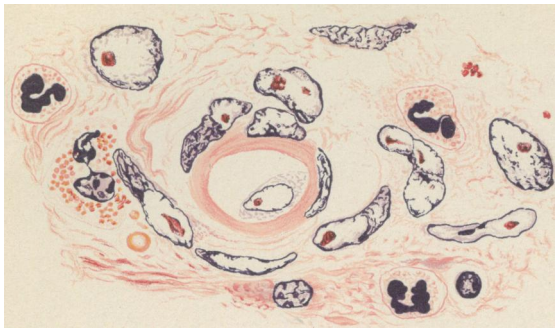

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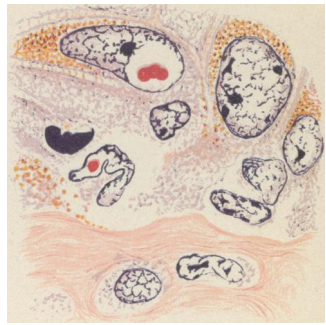
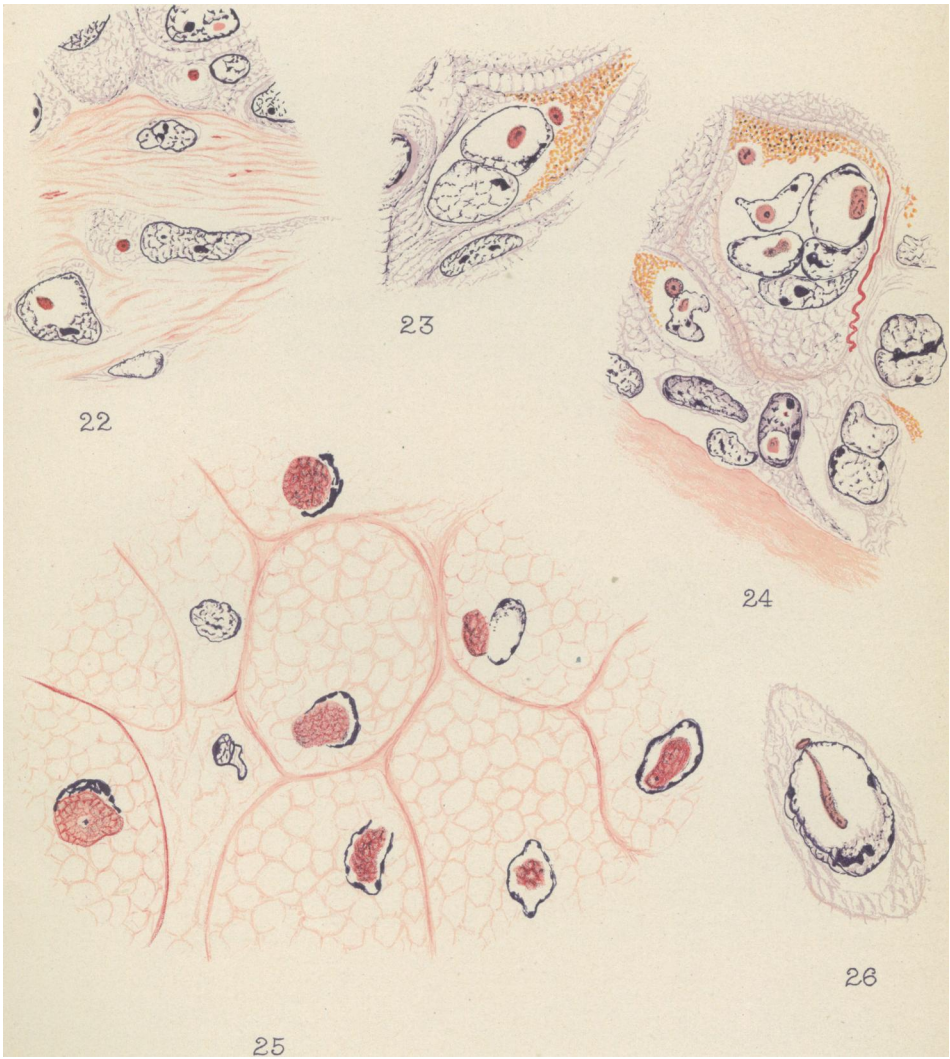
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